Separating Pupae from Larvae

(See also: Sexing larva/pupa)

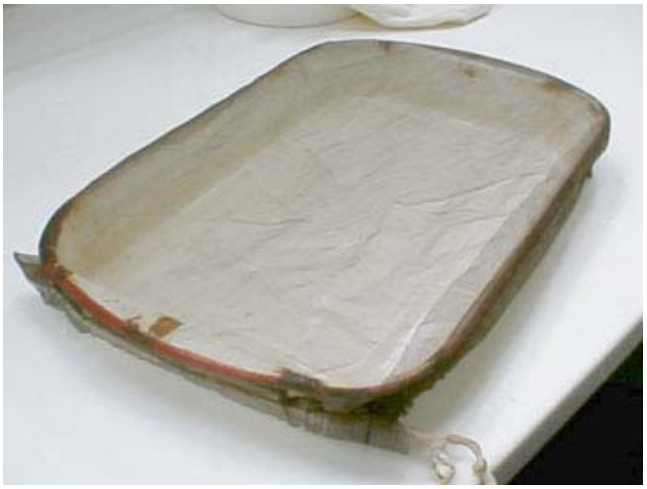
Numerous methods have been developed for separation of pupae from larvae, a process often called 'picking pupae.' Following are a few that work well with many mosquitoes. Surprisingly, the differences in the buoyant density, activity, size, and cold sensitivity means that all methods are not equally useful for various anopheline species, not to mention mosquitoes of other genera.

Four features are used alone or in combination to separate pupae from larvae:

- appearance alone ('hand picking')
- size
- buoyant density
- activity

But first, the easiest way, which requires no picking at all:

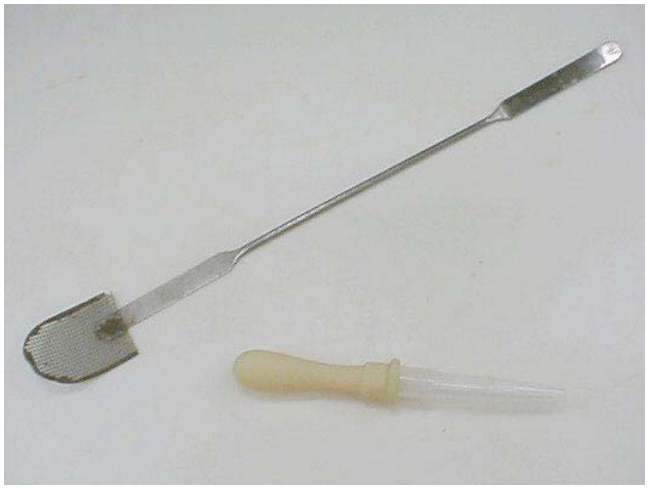
1. Allow them to emerge into the larval culture container.



Shown is a custom-made screen top with elastic and a drawstring around the edge. It is merely stretched over the pan, and the adults emerge and rest on the sides or top. Adults are then collected by aspiration and transferred to a cage. Sugar pads can be placed on top for adult feeding if emergence will occur over a weekend. One can also feed the larvae through the screen. This is a bit messy and many adults die on the surface, but it can get one through a weekend.

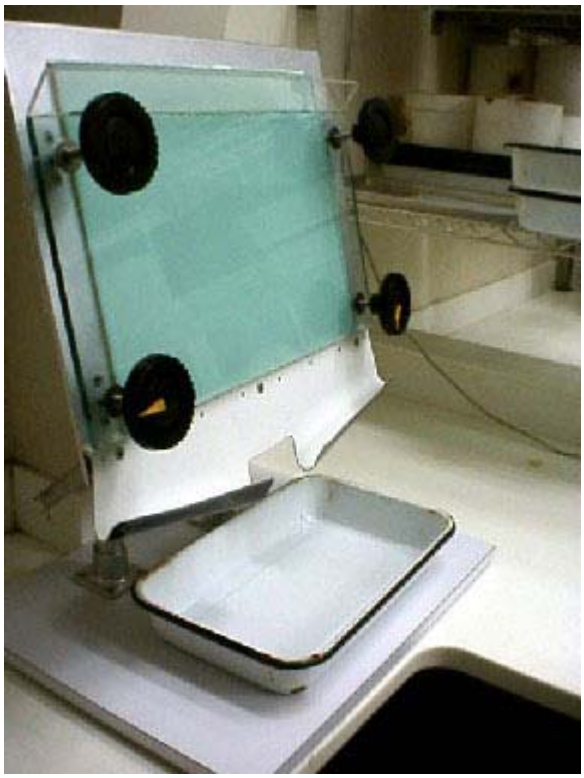
2. Picking pupae 'by hand'...

is the most labor intensive but often best, especially if only a few are present. The basic 'pupa picker' comes in numerous types:



For example: a scoop made of a spoon or spatula and screen, and a disposable P-1000 pipette tip cut off slightly to open the tip with a 2 ml latex bulb attached.

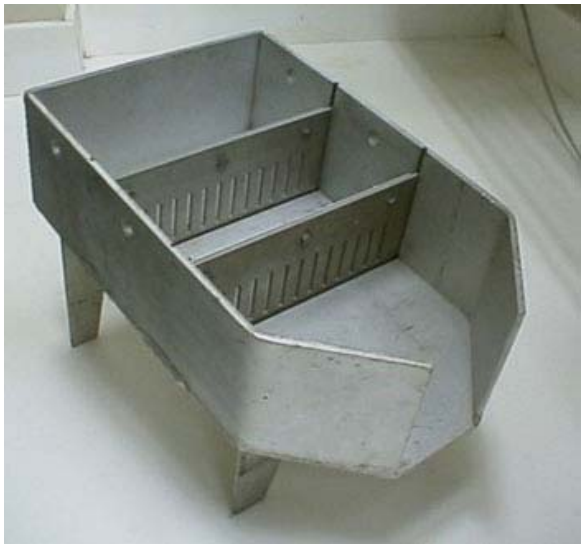
3. Separate them by size



Option A:

The custom-built pupa separator is great for large numbers of one strain, but it requires caution to prevent cross-contamination. The one shown was made by the John Hock company. Two glass plates are adjusted to taper the space between: larger at the top than at the bottom. This allows trapping the pupae (larger) and allowing the larvae (smaller) to flow through. This is also a good way to separate the sexes of pupae that differ considerably between males and females (not *Anopheles spp*.).

Note the arrows on the lower knobs. These knobs are manipulated to adjust the gap and their position should be synchronized. Pouring water into the gap and observing the position and angle of the meniscus is an easy way to get the adjustment close and uniform across the plates. Contamination is the main drawback, and one cannot overload it or congestion with pupae will prevent flow.



Option B:

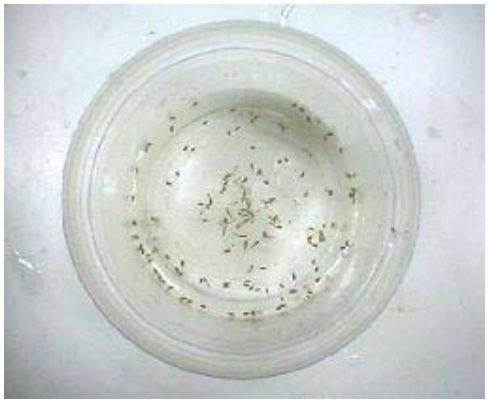
This pupa separator can be effective, but unlike the above design, it requires fine adjustment and very consistently-sized pupae to get good separation. It is almost impossible to tweak on-the-fly for use with numerous strains, so if you choose this route, have numerous devices, each of which is dedicated to one stock. This one is drilled at two places for spray bars (not shown).

4. Separate them by activity / density

Option A:

The vortex method ranges from great to lousy depending on the mosquito. The procedure consists of collecting the pupae and larvae in a strainer and then washing them into a flask with the aid of a funnel. Add water (cool is nice) and swirl as you fill within a breath of the top. Most of the larvae will dive; most of the pupae will rise to the neck. When separation is maximal, pour the pupae off. This method is never 100% effective, so some hand-picking is required. It's effective enough that it often saves lots of time. Shown are *Anopheles gambiae* in the process of separation.

This can also be done in a separatory funnel or an Imhoff funnel, but in these cases, the larvae are drained from the bottom. Cool water will improve the separation, but take care that repeated chilling does not increase mortality.



Option B:

One can get much the same effect by swirling pupae and larvae in a cup and removing the larvae from the middle and / or pupae from the sides. One may combine this with the use of ice water which effectively stuns the negatively buoyant larvae and positively buoyant pupae allowing them to be separated in water without their behavior interfering. However, ice water increases mortality in some strains/species and the time should be minimized. As above, collect the pupae and larvae in a strainer and wash them into a small bowl or separatory funnel containing with ice water. Swirl, wait until separation occurs, and pour the pupae off. In the case of the separatory funnel, open the cock and drain the larvae off into one container followed by the pupae in second. Transfer both back to water at culture temperature. Good method for large numbers and cross-contamination is easily prevented.